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The Lipid Composition and Localization of Free and Esterified Cholesterol in Different Types of Xanthomas

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The lipid composition of 17 xanthomatous lesions from 16 patients with different types of hyperlipoproteinaemia was analyzed. The relative amounts of free cholesterol, cholesterolesters, and phospholipids in xanthomas obtained from patients who subsequently showed regression of their remaining type of xanthomas were different in comparison with xanthomas which did not show regression. One tendinous xanthoma contained more than 50% free cholesterol and resembled in lipid composition the gruel plaques found in atherosclerotic lesions. The relative amounts of free cholesterol, cholesterolesters, and phospholipids in regressing xanthomas were similar to those of fatty streaks found in arteries of children between the ages of 5–10 yr. Histochemical studies using an enzymatic method demonstrated that free and esterified cholesterol were located in focal areas of xanthomatous tissue. It is concluded that the physical state of lipids in xanthomatous lesions can vary remarkably and plays an important role with regard to the possibility of regression.

Xanthomatous lesions can be found in patients with elevated or with normal lipoprotein levels [1,2,3, review 1 and 4].

With the exception of tendinous xanthomas, the other xanthomas are histologically characterized by cholesterolester accumulation in dermal foam cells [5,6]. Accumulated cholesterolester persists in most types of xanthomas (e.g., xanthelasma palpebrarum, xanthoma planum, tendinous xanthoma). However, the accumulated lipids can easily disappear from eruptive xanthomas once elevated serum lipoprotein levels have been lowered [7–10]. Ultrastructural studies have shown that most of the accumulated lipids in eruptive xanthomas as well as in xanthelasma and xanthoma planum are intracellularly localized [11–13]. Moreover, biochemical analyses of tendinous and eruptive xanthomas favored the hypothesis that free cholesterol becomes esterified in both lesions and that during the aging process triglycerides are predominantly degraded in eruptive xanthomas [14,15].

This investigation was performed in order to see if the various types of xanthomatous lesions differ in lipid composition and in the localization of free cholesterol and cholesterolesters.

PATIENTS AND METHODS

Patients

Seventeen xanthomatous lesions were obtained from 16 patients. The lipoprotein abnormalities found in the sera of these patients are shown in Table I.

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Abbreviations:
HDL: high density lipoprotein
IDL: intermediate density lopoprotein
LDL: low density lipoprotein
VLDL: very low density lipoprotein

From one patient 2 xanthelasmas were obtained. Blood sampling and lipid and lipoprotein analyses of the fasting serum was carried out as described [2].

From 9 patients the xanthomatous lesions were removed when no treatment was given. The remaining 7 patients were on treatment for more than 6 mo when the tissue was obtained for lipid analysis (see also Table II).

The treatment for those patients with elevated LDL levels consisted of a low cholesterol, low saturated fat diet with or without the addition of Questran. The patients with elevated VLDL or IDL levels received a low carbohydrate diet and in case the patients were obese the diet was also hypocaloric.

All patients were followed up for more than 1 yr to establish the effect of the treatment schedules on the remaining xanthomatous lesions.

METHODS

Biochemical Analysis of Skin Specimens

Xanthomas were obtained by excision or punch biopsy under local anesthesia and freed of adipose tissue. The specimens were then divided into 2 equal parts. One part was deep-frozen and stored at −20°C for biochemical analysis. The other part was fixed in 4% p-formaldehyde in 0.1 M phosphate buffer (pH 7.4).

The lipids were extracted from minced xanthomas with methanol: chloroform (1:2 v/v) according to Folch et al [18]. After evaporation of the solvent, the lipids were redissolved in 1 ml chloroform. An aliquot was taken for gravimetric determination of total lipids. The lipids were separated by chromatography on thin layers of silicagel and the lipid composition was determined densitometrically after charring with sulfuric acid [19].

Histochemical Analysis

Frozen serial sections (14 μm thick) were cut from unembedded fixed tissue, collected in 0.1 M phosphate buffer (pH 7.4) and washed for 30 min at room temperature to remove any residual fixative. Free and esterified cholesterol were visualized as described in detail elsewhere [20]. In brief, the staining method is based on oxidation of free cholesterol by cholesterol oxidase leading to the release of H_2O_2. Sites where H_2O_2 is produced are visualized by reacting the H_2O_2 with 3-3′diaminobenzidine in a peroxidase catalyzed reaction, which procedure results in the production of a brown insoluble polymer.

Esterified cholesterol can be demonstrated after a preincubation step using cholesterol esterase. Other sections of the xanthomatous tissues were stained with haematoxylin-eosin and with Oil Red 0.

RESULTS

Lipid Analysis

The results of the lipid analysis, arranged according to cholesterolester content as a percentage of total lipids, are shown in Table II. It can be seen that all nine xanthomas obtained from patients who subsequently showed regression had a cholesterolester content of below 35%, and a phospholipid content of more than 25%. In 4 of these 9 xanthomas the triglyceride content was higher than 30%, whereas this relative amount of triglyceride was also found in 2 out of 8 xanthomas without regression. The only xanthoma with a cholesterolester content below 35% which did not show regression, was a tendinous xanthoma which contained an exceptionally high level of free cholesterol. All xanthomatous lesions 9/17 which showed regression, were present on patients with elevated VLDL or IDL levels. No differences in lipid analysis could be observed

TABLE I. *The distribution of various types of xanthomatous lesions*

Type of lesion	No. of patients	Elevated[a] LDL	Elevated[b] VLDL	Elevated VLDL[c] (IDL present)	Normolipaemic
Papulo eruptive xanthoma	5	1	4	—	—
Tubero eruptive xanthoma	5	—	2	3	—
Xanthelasma	1	—	—	—	2+[d]
Tendinous xanthoma	1	1	—	—	—
Xanthoma planum	2	—	—	—	2
Tuberous xanthoma	2	1	—	1	—

[a] LDL = Low Density Lipoprotein; $1.006 < d < 1.063$, elevated = total lipid LDL > 550 mg/dl serum [17] comparable with hyperlipoproteinaemia type IIIA (WHO) [15].

[b] VLDL = Very Low Density Lipoprotein; $0.94 < d < 1.006$, elevated = total lipid VLDL > 260 mg/dl serum [17] comparable with hyperlipoproteinaemia type IV/V (WHO).

[c] IDL = Intermediate Density Lipoprotein, present when the cholesterol/triglyceride ratio in VLDL = >0.5 [10]; VLDL elevated; comparable with hyperlipoproteinaemia type III (WHO).

[d] + = From one patient 2 xanthomas were removed and studied.

TABLE II. *Xanthomas arranged according to relative amount of cholesterolester*[a]

Patients	Type xanthoma	Regression	Lipoprotein elevated	As % of total lipids						Therapy at removal xanthoma	Therapy 1 yr after removal
				TC	FC	CE	PL	TG	FFA		
1	Planum	−	N	51	09	72	01	13	05	—	—
2	Tuberous	−	LDL	51	13	64	16	02	05	Diet	Diet
3a	Xanthelasma	−	N	45	10	60	14	14	02	—	—
3b	Xanthelasma	−	N	44	10	57	16	14	03	—	—
4	Tuberous	−	LDL	43	10	56	17	13	04	Diet + Q	Diet + Q
5	Papulo-eruptive	−	LDL	34	07	45	13	33	02	Diet	Diet
6	Planum	−	N	29	06	39	12	40	03	—	—
7	Tubero-eruptive	+	VLDL	37	18	33	26	21	02	—	Diet
8	Tendinous	−	LDL	72	55	28	13	01	03	Diet + Q	Diet + Q
9	Tubero-eruptive	+	VLDL	34	17	28	32	17	06	—	Diet
10	Tubero-eruptive	+	IDL	32	17	28	31	17	08	—	Diet
11	Tubero-eruptive	+	IDL	34	20	23	45	17	06	—	Diet
12	Tubero-eruptive	+	IDL	35	22	22	38	09	03	Diet	Diet
13	Papulo-eruptive	+	VLDL	21	11	18	23	37	12	—	Diet
14	Papulo-eruptive	+	VLDL	18	08	17	25	42	08	—	Diet
15	Papulo-eruptive	+	VLDL	20	11	15	30	33	11	Diet	Diet
16	Papulo-eruptive	+	VLDL	17	10	12	33	32	13	Diet	Diet

[a] TC = total cholesterol; FC = free cholesterol; CE = cholesterolesters; PL = phospholipids; TG = triglyceride; FFA = free fatty acids; N = normolipaemic; Q = Questran. Total cholesterol was calculated by the equation % TC = 0.58 × % CE + % FC.

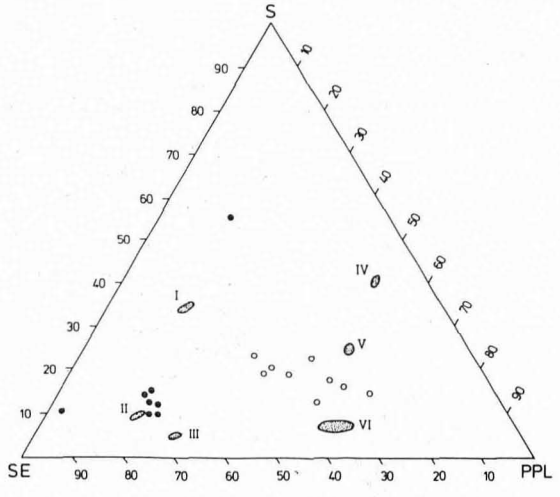


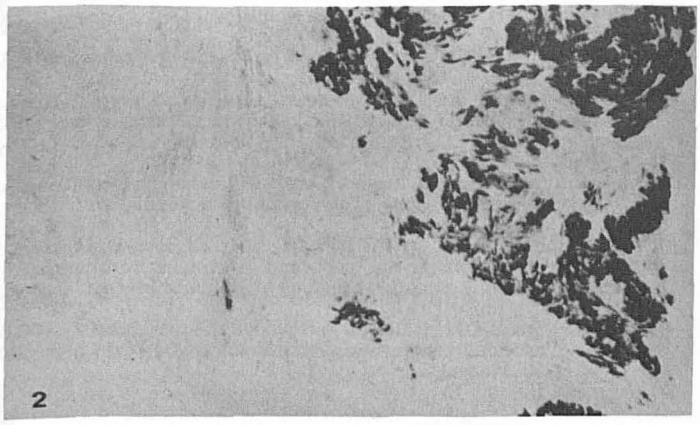
FIG 1. Phase diagram of a 3 component system showing interactions of cholesterol (*S*), phospholipid (*PPL*) and cholesterolesters (*SE*) in excess water at 37°C. ● = xanthomatous lesions without regression; ○ = xanthomatous lesions with regression. *I* represents gruel plaque [23]; *II* represents fatty streak adult [23]; *III* represents adrenal glands [23]; *IV* represents normal intima [24]; *V* represents fatty streak children [24]; *VI* represents high density lipoprotein (HDL) [25]. The tendinous xanthoma at the top of the diagram has an exceptional place and corresponds with a lipid composition of S 57%, SE 39% and PPL 13%.

between the [6] removed prior to treatment and those [13] removed during a dietary treatment.

By plotting the relative amounts of cholesterolesters, free cholesterol and phospholipids, a clear delineation of the several xanthoma types occurred, as shown in a phase diagram (Fig 1) according to Small [21,22]. Those xanthomatous lesions which regressed were clearly separated from the nonregressing xanthomas. We compared our findings with data on the lipid composition of other tissues in which cholesterol or cholesterolesters accumulate (e.g., atherosclerotic vascular lesions, fatty streaks of the vessel wall, adrenal glands) [23]. In this diagram data on the lipid composition of a normal intima, of a fatty streak in a young child [24] and of HDL [25] are also shown. The lipid composition of adult fatty streaks and of the lipid core of atherosclerotic lesions [23] resembles the lipid composition of xanthomatous lesions which do not regress. The lipid composition of fatty streaks in young children [24] and of HDL [25] resembles the findings in xanthomas which regress easily.

Histochemical Observations

In most xanthomatous lesions discreet deposits of cholesterolesters could be found. These deposits were mainly present in the deeper layers of the dermis and the mid-dermis. Free cholesterol could be demonstrated in only 3 lesions which contained more than 20% of free cholesterol. In these lesions the localization of free cholesterol was distinct from the localization of cholesterolesters. This is demonstrated in Fig 2 and 3, showing a positive staining for respectively free cholesterol and



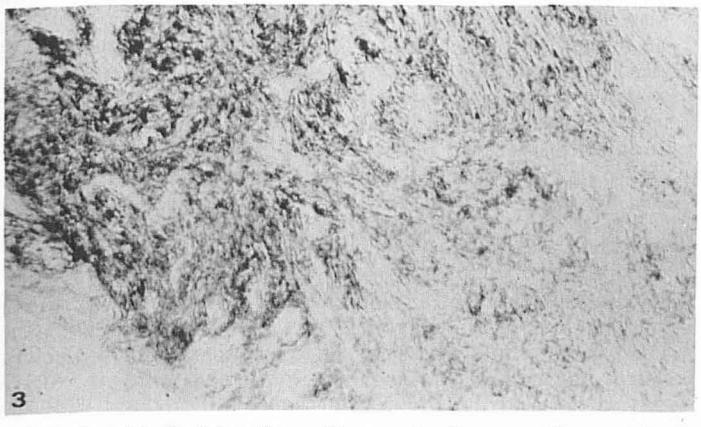


FIG 2 and 3. Serial sections of human tendinous xanthoma stained for free (Fig 2) and esterified cholesterol (Fig 3). Note: different areas of the xanthoma contain free cholesterol or esterified cholesterol. No counterstain (reduced from × 150).

cholesterolesters in different areas of 2 serial sections from a tendinous xanthoma.

DISCUSSION

The role of lipoproteins for the development and regression of xanthomas has been extensively described during the last 10 yr [1,2,26-28]. Also in our study, the patients who showed regression of xanthomas were characterized by elevated VLDL or IDL levels. Biochemical analysis showed that the xanthomas which regressed had a lower cholesterolester and higher phospholipid content in comparison with the xanthomas which did not regress (Table II). However, the triglycerides were elevated in only 4 of the 9 xanthomas obtained from patients who showed a regression of the remaining xanthomas. The lower triglyceride content of several xanthomas with regression could be explained by the "age" of these lesions [14]. As the xanthomas with regression are mainly characterized by a relatively low cholesterolester content and a high phospholipid content the interrelationship between these 2 lipids could be crucial for the possibility of regression. In the phase diagram, the interrelationship of free cholesterol, phospholipids and cholesterolesters are shown and a further deviation between the different groups of xanthomas is obtained (Fig 1). According to the physical studies of Small [21] the relative amounts of phospholipids, cholesterolesters, and free cholesterol will determine the physical properties of these lipid mixtures. Using the data of Small [21,22], the xanthomas which regressed easily are characterized by an abundance of phospholipid-liquid crystalline phase and less cholesterolester-liquid or liquid-crystalline phase. In the other group of xanthomas the physical state of the lipids is the reverse.

The melting point of the cholesterolesters will determine whether these lipids are accumulated in a liquid-crystalline or liquid phase. Most cholesterolesters in xanthomatous tissue are composed of cholesterol oleate (melting point ± 50°C) [29] and it is therefore conceivable that the cholesterolesters are predominantly accumulated in a liquid-crystalline phase [21].

The tendinous xanthoma has an exceptional lipid composition mainly due to the high amount of free cholesterol. This xanthomatous lesion resembles the core of advanced gruel plaques and should be characterized by the presence of cholesterol-monohydrate crystals [30] as confirmed histochemically and electronmicroscopically [31].

The histochemical observations in the xanthomatous lesions show that the cholesterolesters are mainly accumulated in focal areas. These findings are in accordance with the localization of foam cells as observed by light- and electron microscopy [5,6]. Moreover, the histochemical studies in the tendinous xanthoma showed that free cholesterol and cholesterolesters are located in different areas. It is therefore understandable that certain specimens of tendinous xanthoma might contain excessive amounts of free cholesterol whereas other specimens do not [14,15]. The biochemical and histochemical data on the various types of xanthomatous lesions all suggest that no regression occurs if focal accumulated cholesterolesters are present in a liquid-crystalline phase. The same holds true for the accumulated cholesterol crystals in the tendinous xanthomas. However, when the xanthomas have a phospholipid-liquid crystalline-like phase regression can easily occur. Our study suggests that apart from lipoprotein levels also the physical state of the lipids in xanthomatous lesions is an important factor with regard to the possibility of regression.

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